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Experimental increase of testosterone increases boldness and decreases anxiety in male African striped mouse helpers

Julien Raynaud[1], Carsten Schradin[1,2,3,4]

[1] Institute of Evolutionary Biology and Environmental Studies, University of Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland.

[2] Université de Strasbourg, IPHC-DEPE

[3] CNRS, UMR7178, 67087 Strasbourg

[4] School of Animal, Plant and Environmental Sciences, University of the Witwatersrand, Private Bag 3, Wits 2050, Johannesburg, South Africa.

Correspondence to be sent to: Julien Raynaud, Animal Behavior Group, Institute of Evolutionary Biology and Environmental Studies, University of Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland.

e-mail: julien.raynaud@ieu.uzh.ch

Phone: 0041 (0)44 635 52 85

Fax: 0041 (0)44 635 54 90

Abstract

Males of many species can adjust their behaviors to environmental conditions by changing reproductive tactics. Testosterone surges in adult breeding males typically inhibit the expression of paternal care while facilitating the expression of aggression during environmental changes. Similarly, in non-breeding philopatric males of cooperatively breeding species, up-regulation of testosterone may inhibit alloparental care while facilitating dispersal, i.e. males might become bolder and more explorative. We tested this hypothesis in philopatric male African striped mice, *Rhabdomys pumilio*. Striped mouse males can either remain in their natal group providing alloparental care or they can disperse seeking mating opportunities. Compared to philopatric males, dispersed males typically show higher testosterone levels and lower corticosterone levels, and more aggression towards pups and same sex conspecifics. We experimentally increased the testosterone levels of philopatric males kept in their family group when pups were present. Testosterone-treated males did not differ significantly from control males in alloparental care and in aggression toward same-sex conspecifics. Compared to control males, testosterone treated males were bolder, more active, less anxious; they also showed lower corticosterone levels. Philopatric males were sensitive to our testosterone treatment for dispersal- and anxiety-like behavior but insensitive for social behaviors. Our results suggest a role of testosterone in dispersal.

Keywords: social flexibility, strategy, cooperative breeding, exploration, shyness, dispersal, solitary-living, roamer.

Introduction

The ability of an individual to change its reproductive tactic as a response to changes in the social and non-social environment can provide numerous advantages (Lott 1991). In several species, males unable to compete with larger males with greater competitive abilities adopt alternative reproductive tactics (ARTs) (Gross 1996; Taborsky 1997; Oliveira et al. 2005). ARTs are discontinuous behavioral and other traits selected to maximize fitness in two or more alternative ways (Oliveira et al. 2008). If the environment and the individual body conditions do not favor dispersal, males of social species can remain in their natal group as philopatric helpers providing care toward the breeder's offspring (i.e. alloparental care), instead of dispersing and seeking mating opportunities (Schradin et al. 2012b). Males are predicted to disperse and follow reproductive tactics with higher fitness when certain conditions change, e.g. their environment or their competitive ability (Schradin & Lindholm 2011). For instance, a decrease in population density enhances dispersal in philopatric African striped mice, *Rhabdomys pumilio* (Schoepf & Schradin 2012b). Dispersal implies essential behavioral shifts, i.e. decreased alloparental care and increased behaviors facilitating dispersal. However, less is known about the proximate mechanisms mediating these adaptive behavioral changes which are thought to rely on hormone-based mechanisms (Moore 1991; Moore et al. 1998).

Androgen levels, for instance testosterone, rise during puberty and correlate with dispersal in many species (Nelson 2005). In Belding's ground squirrels, *Spermophilus beldingi*, early testosterone exposure caused inter-individual dispersal differences (Holekamp et al. 1984; Nunes et al. 1999). However, no study has demonstrated that

testosterone surges cause dispersal at later life history stages (e.g. sexual maturity). Yet, dispersal is a risky undertaking (Metzgar 1967; Wolf 1994; Solomon 2003) and the anxiolytic effect of testosterone (Aikey et al. 2002) may facilitate dispersal. Similarly, ~~suggested that~~ testosterone may cause dispersal through the facilitation of exploratory behavior (Holekamp et al. 1984). In African striped mice, ~~Raynaud et al~~ (Raynaud et al. 2012) ~~showed that~~ testosterone-treated juvenile males expanded their home ranges and showed decreased corticosterone levels (Raynaud et al. 2012). As the final decision to disperse may rely on ecological factors (population density and reproductive competition) (Schradin et al. 2010b; Schradin & Lindholm 2011; Schoepf & Schradin 2012b), an increase of testosterone levels may facilitate dispersal through both the inhibition of anxiety-like behavior and a decrease in glucocorticoid levels.

Changes in testosterone levels may also be a mechanism mediating the expression of alloparental care in cooperatively breeding species (Schoech et al. 2004). Elevated testosterone levels typically decrease paternal care in birds (Wingfield et al. 1990), although this testosterone action seems more species specific in mammalian fathers (Hirschenhauser & Oliveira 2006; Storey et al. 2006). ~~Prolactin levels correlate positively with alloparental care in different species, such as the Florida scrub jay, *Aphelocoma c. coerulescens* (Schoech et al. 1996). However, in other species, helpers show both low prolactin and low testosterone levels, such as the African striped mouse (Schradin & Yuen 2011). This suggests that low testosterone levels may facilitate alloparental care instead of high prolactin levels.~~ In prairie voles, *Microtus ochrogaster*, ~~Roberts et al~~ (Roberts et al. 1996) ~~demonstrated that~~ testosterone administrated postnatally decreased the alloparental responsiveness of males (Roberts et al. 1996). Thus, while up-regulation

Kommentar [CS1]: I do not think this is necessary

of the hypothalamus pituitary gonadal axis (HPG) resulting in increased testosterone levels may facilitate dispersal-like behavior, high testosterone levels may result in a decrease of alloparental care. Consistent with this hypothesis, ~~in~~ meerkats male helpers, *Suricata suricata*, showed high testosterone levels and decreased pup feeding rates after prospecting for dispersing opportunities (Young et al. 2005). However, experimental demonstration of the role of testosterone in mediating both behaviors - dispersal and alloparental care - is lacking.

In the present study, we tested the role of testosterone in social behavior (alloparental care, affiliative and aggressive behaviors) and in non-social behavior (boldness, exploration, and anxiety-like behavior), which may facilitate dispersal, in the African striped mouse. Males striped mice can follow one of three ARTs, accompanied by changes in hormonal profile and parental care: 1) philopatric group-living males showing the lowest testosterone levels, highest corticosterone levels and alloparental care; 2) solitary-living roamers showing the highest testosterone levels, low corticosterone levels and no parental care; and 3) social dominant group-living territorial breeders showing intermediate testosterone levels, low corticosterone levels and high parental care (Schradin & Pillay 2003; Schradin et al. 2009b). Juvenile males can disperse or alternatively they can remain in their natal group to disperse at a later stage as adult (Schradin & Pillay 2005). Philopatric group-living males have to disperse from their natal group to become either solitary-living roamers or dominant group-living territorial breeders (Schradin et al. 2009b; Schradin & Lindholm 2011). Testosterone levels increased when philopatric group-living males changed into solitary-living roamers (Schradin & Yuen 2011). Furthermore, ~~Schoepf and Schradin~~ (Schoepf & Schradin

2012a) ~~demonstrated that~~ philopatric males became more aggressive toward same-sexed conspecifics and pups after they dispersed (Schoepf & Schradin 2012a). These studies suggest that an increase of testosterone levels may inhibit the expression of alloparental care and enhance the expression of aggressive behavior. As dispersing males showed increased testosterone and decreased corticosterone levels (Schoepf and Schradin, 2013), we hypothesize that an increase of testosterone levels may cause philopatric group-living males to be bolder (i.e. more prone to undertake risky behavior (Wilson et al. 1994)), more explorative (i.e. more prone to approach a novel object (Powell et al. 2004)), and less anxious (i.e. more prone to spend time in the anxiogenic open arms than in the safer closed arms of an elevated plus maze (Pellow et al. 1985)). We referred these different behaviors as "dispersal-like behavior". We tested these predictions under standardized conditions in captivity by experimentally increasing testosterone levels of philopatric group-living males.

Kommentar [J2]: animal behaviour

Materials and methods

Animals and breeding conditions

The founder pairs of the striped mouse colony housed at the University of Zurich originated from individuals trapped in the Succulent Karoo in South Africa in 2002. Mice were housed under a 11:13 h light / dark regime with partly controlled temperature (approx. 23ºC). Family groups were housed in two glass tanks (50x30x30cm) which were connected to one another with a flexible plastic tube. Additionally, one plastic cage (20x13x15 cm) was provided with nesting material. All tanks and cages had 5 cm of wood shavings for bedding. Each mouse received a 4 g seed mixture in the morning, a

piece of fruit or lettuce at midday (1 g/mouse), and 2 mealworms in the afternoon. Water was provided *ad libitum*.

We used 11 family groups consisting of one dominant group-living territorial breeder, one breeding female and two litters. Philopatry was mimicked by leaving offspring in the family cage. Several previous studies demonstrated that captive philopatric group-living males show similar hormonal profiles in comparison to males studied in field conditions +, with philopatric males showing lower testosterone and higher corticosterone levels than solitary kept males, becoming sexually mature at an earlier age, developing larger testes and producing more sperm (Schradin et al., 2009b; Schradin et al., 2012a; Schradin et al., 2013). When the offspring of their first litter were 21 days old, the litter was reduced in number to one philopatric group-living female and two philopatric group-living males. Each mouse was dyed for identification with a unique mark on the pelage (Rapido, Pinetown South Africa).

Experimental testosterone manipulation

We started the testosterone treatment on the day of birth of the second litter when philopatric group-living males of the previous litter were 36.0 ± 2.4 days old. Philopatric group-living males were anesthetized with ether and implanted subcutaneously behind the neck using a precision trochar 10 gauge (Innovative Research of America, Sarasota, FL, USA). In each family, one of the two males randomly received one pellet of 3.5 mg testosterone (time-release pellets from Innovative Research of America, Sarasota, FL, USA) referred to as "test male" while his same-litter sibling received an empty pellet (placebo) referred to as "control male".

157

158 *Blood collection*

159 Blood samples were collected in the morning within one hour after the lights went on to
160 control for a possible circadian rhythm of hormone secretions. Mice were anaesthetized
161 with ether and a blood sample of 200µl was collected from the sub-lingual vein (Heimann
162 2006) within less than three minutes. After one hour, blood samples were centrifuged two
163 successive times for 10 min. The resulting serum was frozen in aliquots of 50 µl for
164 testosterone, and 10 µl for corticosterone assays until used. Blood samples were collected
165 from each test and control male directly before the implantation (D0), a day after the
166 implantation (D1), nine days after the implantation (D9) and 14 days (D14) after
167 implantation.

168

169 *Reproductive status and body mass monitoring*

170 Reproductive status (scrotal, i.e. testes fully descended, or non-scrotal) and body mass (in
171 grams) of the test and control males were recorded after each blood sample was taken.

172

173 *Breeder aggression*

174 In African striped mice, breeding males and females could show aggressive behaviors
175 towards the test males that could influence the expression of alloparental care. We daily
176 recorded for 30 minutes the frequency of aggressive behaviors (i.e. chasing, fighting, and
177 biting) of breeding males and females toward both the test and control males during the
178 whole experiment.

179

Kommentar [J3]: I removed the sentence: "In Mongolian gerbils high testosterone levels can trigger the expulsion of philopatric males from their families"

Alloparental care

We performed daily alloparental care observations from D0 until D9. Observations were alternatively performed during the morning (between 9:00 am and 12:00 am) and the next day during the afternoon (between 03:00 pm and 06:00 pm) to cover alloparental care observations during the whole active period of African striped mice. Nests were videotaped for 30 min without any observers inside the animal room. The first five minutes of each video were ignored to minimize any effects of a potential disturbance as a result of the initial camera set-up. Using the software EthoLog 2.25© (Ottoni 2000), we recorded the time spent (in seconds) by each test and control male in the nest, the time spent huddling, and the time spent licking the pups. We also recorded the frequency of carrying pups in the mouth, and retrieving the pups. We considered the total amount of alloparental care provided by each test and control male as the sum of the time spent in the nest, huddling and licking the pups. We finally considered the mean per day (%/day) of huddling, licking the time spent in the nest, and the total amount of alloparental care (i.e. huddling + Licking + time spent in the nest) for statistical analyses.

Behavioral tests

On D10, we performed three successive behavioral tests in the same order for every test and control mouse (see above). ~~Yuen demonstrated that~~ Tthe order of these tests had no significant influence on boldness, activity, exploration and aggression in male African striped mice (unpublished data).

1- Boldness assessment: open field test

The subject was placed in the periphery of a neutral test arena (80x40x60cm made of wood), and observed for five minutes. The time that mice stay close to the wall (thigmotaxis) and activity were used as an indicator of boldness / shyness: low thigmotaxis and high activity indicate an increase of boldness (Sneddon 2003). Thus, the amount of time the mouse spent with at least half a mouse length away from the arena's walls was recorded to assess increased boldness. We also measured the mouse activity. For this, we recorded every 15 seconds whether the mouse was moving (i.e. walking) or immobile in the open area.

2- Exploratory assessment: novel objects test

With the subject inside the same test arena, two novel objects (a fixed and a movable object: rubber tiger and table tennis ball) were placed at the opposite end of the arena. Direct observations were performed during 5 min to record latency to approach (seconds) and sniffing the object (frequency).

3- Aggressiveness assessment: dyadic encounters

Same sex encounter tests were performed in the same test arena. At the beginning, a partition in the middle divided the arena in two compartments. At one side a stimulus animal was placed. Stimulus animals ~~in these tests~~ were males (22-40 days old) housed in same-sex sibling groups consisting of 2 to 3 brothers from the same litter. They were removed from their family when 16-21 days old and they were not genetically related to the focal males. In all cases, the focal animals (i.e. test and control males) were bigger than the stimulus animals (38.1 ± 2.6 vs. 22.1 ± 2.1; N = 16; V = 171; $p < 0.001$), as it is

known that dominance is weight related in striped mice (Schradin 2004). The focal animal (i.e. test or control males) was placed on the other side. After a habituation period of 5 min, the partition was removed and the focal animal was observed for 15 min. No damaging fights occurred during any dyadic encounters. The frequency of aggressive behaviors (chasing, fighting, and biting) were recorded. We also recorded the time spent in body contact and the frequency of sniffing and grooming the stimulus animal. This test has been used previously to measure aggression in striped mice from the field (Schradin et al. 2010a) and in captivity (Schradin, unpublished data).

4- Anxiety assessment: elevated plus maze

Test and control males were tested in an elevated plus maze on D12. The elevated plus maze consisted of two anxiogenic open arms and two safe enclosed arms with an open roof, arranged such that the open arms were opposite to each other. The maze was elevated to a height of 100 cm. We videotaped the number of entrance into each arm and the duration of visits inside each arm during 5min. Two indices were used to measure the aversiveness of the open arm: ratio of open arm entries to total arm entries (OER) (Handley & Mithani 1984) and the ratio of time spent in open arms to total time spent in all arms (OTR) (Pellow et al. 1985). The activity of the mice was evaluated using the total arm entries during the trial.

Hormone assays

We performed testosterone and corticosterone assays with commercial kits (IBL Hamburg, Germany), previously validated for striped mice serum (Schradin 2008a).

Since corticosterone levels are very high in philopatric group-living males (Schradin et al. 2009b), samples for the corticosterone assay were diluted (2: 48) with the zero standard. For three samples of testosterone, the amount of serum aliquots was too small for hormone assay and was thus diluted (1: 1) with the zero standard. The intra-assay coefficient of variation was 8.98 % for testosterone and 14.84 % for corticosterone.

Data analysis

We stopped the experiments in two families because we observed wounds in test and control males, and these males ~~did not have access to water and food for two consecutive days. These males~~ had to be~~were~~ removed from their family units and euthanized. This ~~finally~~ reduced the sample size down to nine. Furthermore, in one family, the test male died after the third blood sample (D9). Thus, for this experiment, we did not obtain a last blood sample (D14) and we could not collect behavioral data about boldness, exploration, aggression, and anxiety.

Statistical analyses were carried out with R 2.15.0 . Results are presented as mean ± SEM and significance was accepted at $\alpha \leq 0.05$. We used non-parametric statistical analyses due to small sample sizes. Each pairwise comparison (between test and control males) was performed with paired exact Wilcoxon Signed Rank Tests and Fisher's Exact Test. To test for relationships of boldness and anxiety with activity, we performed Spearman rank correlations.

Results

Serum hormone levels

Before the treatment, test and control males did not significantly differ in testosterone levels (0.97 ± 0.21 ng/ml *vs.* 0.85 ± 0.15 ng/ml; N = 9; V = 29 p = 0.50; Figure 1a). On D1, D9, and D14, test males showed significantly higher testosterone levels than control males (D1: 47.08 ± 3.97ng/ml *vs.* 1.88 ± 0.60 ng/ml; N = 9, V = 45, p = 0.004; D9: 20.32 ± 3.45 ng/ml *vs.* 1.82 ± 0.59 ng/ml; N = 9, V = 45, p = 0.004; D14: 18.64 ± 5.13 ng/ml *vs.* 1.64 ± 0.24 ng/ml; N = 8, V = 36, p = 0.008).

Before the treatment, test and control males tended to differ in corticosterone levels (876.78 ± 118.49 ng/ml *vs.* 510.49 ± 100.69 ng/ml; N = 8; V = 3; p = 0.08; Figure 1b). On D1 and D9, test males showed significantly lower corticosterone levels than control males (D1: 517.97 ± 100.40 ng/ml vs. 1005.13 ± 251.80 ng/ml; N = 9; V = 6; p = 0.05; D9: 331.39 ± 31.82 ng/ml *vs.* 963,23 ± 218.41 ng/ml; N = 9; V = 5; p = 0.04). On D14, test males tended to show lower corticosterone levels than control males (D14: 259.65 ± 38.14 ng/ml *vs.* 971.38 ± 274.44 ng/ml; N = 7, V = 3, p = 0.08).

Reproductive status and body mass

Test males did not differ from control males in body mass before and during the testosterone treatment (D0: 28.92 ± 3.47 g *vs.* 27.94 ± 3.08 g; N = 9, V = 25, p = 0.82; D1: 31.47 ± 3.53 g *vs.* 30.28 ± 3.07 g; N = 9, V = 31, p = 0.34; D9: 34.67 ± 2.34 g *vs.* 34.89 ± 2.76 g; N = 9, V = 20, p = 0.82; D14: 34.03 ± 3.16 g *vs.* 34.60 ± 3.17 g; N = 8, V = 14, p = 0.64). Test males did not differ from control males in reproductive status before and during the testosterone treatment, as most males were scrotal already on D0 (89 % *vs.* 78 %; Fisher's Exact Test: N = 9, p > 0.99), D1 (89 % *vs.* 89 %; Fisher's Exact Test: N =

9, p > 0.99), D9 (100 % *vs.* 89 %; Fisher's Exact Test: N = 9, p > 0.99), and all test and control males were scrotal on D14.

Breeder aggression

We never observed breeding males and females biting either test or control males. Fighting occurred in one out of nine families and did not differ significantly between test and control males (0.2 ± 0.2 *vs.* 0; N = 9; V = 1; p > 0.99). Chasing occurred in three out of nine families and did not differ significantly between test and control males (2.7 ± 2.7 *vs.* 0.8 ± 0.5; N = 9; V = 3; p > 0.99).

Kommentar [CS4]: What about the two families where the males had to be removed and euthanized?

Alloparental care

The percentage of time that males showed alloparental care did not differ between test males and control males (15.40 ± 5.77 % *vs.* 5.92 ± 2.26 %; N = 9; V = 34; p = 0.20; Figure 2). Test males tended to huddle the pups longer than control males (12.43 ± 5.23 % *vs.* 3.90 ± 2.30 %; N = 9; V = 38.5; p = 0.07) while there was no difference for the percentage of licking (0.29 ± 0.14 % *vs.* 0.12 ± 0.06 %; N = 9; V = 15; p = 0.40) and no difference for the percentage of time spent inside the nest (2.69 ± 1.43 % *vs.* 1.89 ± 0.87 %; N = 9; V = 19; p = 0.94).

Boldness, activity (open field test)

Test males tended to spend more time away from the wall than control males (52.25 ± 14.77 s *vs.* 26.00 ± 9.61 s; N = 8; V = 31; p = 0.08; Figure 3a). Test males were significantly more active than control males (61.25 ± 10.80 % *vs.* 38.75 ± 8.80 %; N = 9;

V = 32.5; p = 0.05; Figure 3b). Both for the test and control males, the time spent away from the wall was significantly positively correlated with activity (test males: r_s = 0.92, p = 0.001; control males: r_s = 0.88, p = 0.004).

Exploration (novel object test)

Neither the latency to approach the fixed nor to approach the mobile object ~~did~~ differed between test and control males (fixed object: 101.13 ± 34.05 s *vs.* 148.13 ± 45.02 s; N = 8; V = 8; p = 0.35; mobile object: 118.88 ± 33.97 s *vs.* 147.75 ± 46.07 s; N = 8; V = 10; p = 0.31). Test males did neither~~not~~ sniff the fixed nor the mobile object more often than did control males (fixed object: 8.50 ± 2.49 *vs.* 4.50 ± 1.89; N = 8; V = 17; p = 0.21; mobile object: 7.38 ± 1.80 *vs.* 4.75 ± 1.57; N = 8; V = 29; p = 0.14).

Aggression (dyadic encounter)

The frequency of aggressive behaviors by test and control males toward the stimulus animal did not differ significantly (2.00 ± 0.82 *vs.* 1.63 ± 0.84; N = 8; V = 10; p = 0.59). The time spent in body contact with the stimulus animal did not differ significantly between test and control males (42.63 ± 34.83 s *vs.* 27.63 ± 20.09 s; N = 8; V = 17; p = 0.94). There were no significant differences between test and control males for the ~~nor did the~~ frequency of sniffing (8.50 ± 2.35 *vs.* 7.50 ± 2.99; N = 8; V = 17; p = 0.94) and for the frequency of grooming the stimulus animal (0.88 ± 0.52 *vs.* 0.63 ± 0.38; N = 8; V = 6; p = 0.86).

Anxiety (elevated plus maze)

Test males spent more time in the open arm (OTR) than control males (39.82 ± 10.34 % *vs.* 19.78 ± 9.06 %; N = 8; V = 28; p = 0.02; Figure 3c). The open entry ratio (OER) did not differ significantly differ between test and control males (36.85 ± 7.58% *vs.* 32.72 ± 7.26 %; N = 8; V = 16; p = 0.29). Test males tended to be more active (total arm entries) than control males (16.88 ± 3.24 *vs.* 8.00 ± 2.12 N = 8; V = 32; p = 0.06; Figure 3d). The OTR was significantly positively correlated with total arm entries for control males (r_s= 0.83, p = 0.01) but not for test males (r_s= 0.19, p = 0.66).

Discussion

Up-regulation of testosterone can decrease the expression of alloparental care (Roberts et al. 1996) and may facilitate dispersal (Schoech et al. 2004). In many cooperatively breeding species, juvenile males reaching puberty can either stay in their natal group as helpers showing alloparental care, low androgen and glucocorticoid levels, or they can disperse, which is usually positively correlated with an increase of testosterone levels (Schoepf and Schradin, 2013). However, the extent to which testosterone influences these behavioral changes is still debated (Lynn 2008; Gleason et al. 2009). In the current study, we demonstrated that an experimental increase of testosterone levels in philopatric group-living males increase activity, boldness and decrease anxiety, i.e. traits that may facilitate dispersal (Holekamp et al. 1984). However, we found no evidence that increased testosterone levels enhance aggressive behavior ~~n~~or decrease the expression of alloparental care.

The 3.5 mg testosterone pellets increased serum testosterone levels above the maximum physiological levels measured in field samples (15 ng/ml; Schradin

363 unpublished data). This was unexpected as in other rodent species of similar body mass
364 and age, 5 mg testosterone doses resulted in a much lower increase of testosterone levels
365 (in Syrian hamsters: increase from 1ng/ml to 4ng/ml (Romeo et al. 2003); in castrated
366 adult male Rockland-Swiss albino mice: increase to 4.5ng/ml (Barkley & Goldman
367 1977). One hypothesis might be species differences in the metabolism of testosterone, i.e.
368 a higher conversion rate of testosterone into its metabolites in Syrian hamsters and wild
369 house mice than in African striped mice. As we induced supra-physiological testosterone
370 levels, our results have to be discussed cautiously. A physiological increase of
371 testosterone levels might have had different effects, which could explain why we did not
372 find an effect on aggression and allo-parental care. Importantly, we found the expected
373 effects on dispersal related behaviors, indicating that our supra-physiological testosterone
374 levels did not induced unresponsiveness to testosterone, for example via reduced
375 androgen or estrogen receptor expression. However, aggression, allo-parental care and
376 dispersal related behaviors are regulated by different brain areas that might respond
377 differently to very high testosterone levels In conclusion, while very high testosterone
378 levels only influenced dispersal like behaviors, it is currently unknown what effect
379 physiologically relevant levels would have had on aggression and on allo-parental care.
380 In African striped mice, both paternal group-living territorial breeders and non-
381 paternal solitary-living roamers display high testosterone levels (Schradin & Pillay 2003;
382 Schradin et al. 2009b) but differ in prolactin levels (Schradin 2008b), indicating that
383 prolactin may mediate paternal care (Schradin 2007; Wynne-Edwards & Timonin 2007).
384 However, African striped mouse philopatric males show low prolactin levels which
385 indicates that alloparenting is mediated by different mechanisms (Schradin 2008b). Our

386 results show that test males did not show significantly less alloparenting than control
387 males. Interestingly, test males even tended to huddle pups more often than control
388 males. Our results are surprising because free-ranging dispersing males showing high
389 testosterone levels are more aggressive towards pups than non-dispersing philopatrics
390 males showing low testosterone levels (Schoepf & Schradin 2012a; 2013). As exogenous
391 testosterone alters alloparenting in other mammal species, for instance, in prairie voles
392 (Roberts et al. 1996), our results support the hypothesis that testosterone effects on
393 parental care are species specific in mammals (Storey et al. 2006).

394 Breeding males and females showed very few aggressive behaviors toward male
395 helpers, and they did not show more aggression towards test than control males. This
396 suggests that the behavior of breeders did not influence the display of alloparental care in
397 our study. Our results also suggest that test males might be insensitive to increased
398 testosterone levels during the experiment. Similarly, exogenous testosterone did not
399 reduce paternal care in Puerto Rican frogs, *Eleutherodactylus coqui* (Townsend et al.
400 1991) and in Chestnut-collared longspurs, *Calcarius ornatus* (Lynn et al. 2002). The
401 behavioral insensitivity to testosterone may also explain why test and control males did
402 not differ in aggressive behavior. Thus, our results might be either due to the non-
403 readiness of the brain to respond to testosterone signals (Lynn 2008) or to the adverse
404 effects of testosterone supra-physiological levels produced by 3.5mg testosterone pellets,
405 for example, by downloading androgen receptors (Handa et al. 1994) or estrogen receptor
406 α, which are important in the regulation of social behavior (Cushing et al. 2008).

407 While we found no significant effect of testosterone on alloparental care and
408 aggression, we observed significant changes in activity, boldness, and anxiety-like

behaviors. Test males tended to be bolder and also significantly more active than control males during the open-field tests. While boldness was significantly and positively correlated with activity, test males also spent significantly more time in the open arms of the elevated plus maze regardless of their level of activity (i.e. total arm entries). Thus, increased testosterone levels increase boldness and this indicates a significant anxiolytic effect of testosterone in captive philopatric male African striped mice. These results contrast with the non-conclusive effects of testosterone on alloparenting and aggression. Activation of androgen receptors in the hypothalamus is necessary to reduce anxiety in mice and rats (Zuloaga et al. 2008; Zuloaga et al. 2011). If the supra-physiological levels of testosterone had down-regulated androgen receptors, reducing brain responsiveness to testosterone, we would expect no significant difference in anxiety-like behavior between test and control males.

Test males showed significantly lower corticosterone levels than control males. Our result indicates that an increase~~up regulation~~ of testosterone decreases the basal corticosterone levels of philopatric males. Interestingly, test males also showed lower anxiety-related responses during the elevated plus maze than control males. In laboratory mice, it is well known that lower basal corticosterone levels decrease the reactivity of the hypothalamus-pituitary-adrenal (HPA) axis (Touma et al. 2008). In African striped mice,~~The~~ lower basal corticosterone levels induced by the increase of testosterone~~of test males~~ levels might be related to a decreased stress response, for example during the open-field test and elevated plus maze test. In other words, t~~This suggests that~~ changes in serum testosterone levels might mediate~~s~~ the reactivity of the HPA axis, which could influence how philopatric males cope with stressful situations such as dispersal (as

described for belding ground-squirrels; Holekamp et al. 1984). ~~In laboratory mice, lower basal corticosterone levels decrease the reactivity of the hypothalamus pituitary adrenal (HPA) axis (Touma et al. 2008), and African striped mouse males kept alone showed lower basal corticosterone levels than males kept with their family (Schradin et al. 2009a). Interestingly, African striped mouse males kept with their family show greater stress response than juvenile males separated from their family and kept individually: these mice showed higher increased corticosterone levels after an elevated plus maze trial and increased anxiety (unpublished data). Overall, our results suggest that testosterone mediates the reactivity of the HPA axis, influencing how philopatric males are coping with stressful situations.~~

An important question is whether it is the changes in hormones that drive behavioral changes to disperse in free ranging individuals, or whether it is the decision to disperse that drives the changes in hormone levels, or a combination of both? At present, we cannot fully answer these questions. In an experimental field study it was shown that males showed an increase of testosterone levels and a decrease of corticosterone levels after they dispersed, but it was not known whether this change occurred before or after dispersal (Schoepf and Schradin, 2013). However, males that had dispersed in this field experiment were already scrotal before dispersal, while males that remained philopatric were often not (Schoepf & Schradin 2012a), indicating that physiological changes might have been initiated before dispersal (Schoepf & Schradin 2013). From studies in captivity we know that the testes of philopatric males are as good in producing testosterone as those of solitary males, indicating that philopatrics could quickly increase testosterone levels (Schradin et al 2012a). Our present study gives support to the hypothesis that

455 changes in hormones induce behavioral changes, as experimental increase of testosterone
456 increased dispersal like behaviors, which would increase the likelihood of dispersal in the
457 field. Under field conditions, male philopatrics with experimentally increased
458 testosterone levels respond similarly, by decreasing their corticosterone levels and by
459 increasing their home ranges, indicating that they explore possibilities for dispersal
460 (Raynaud et al. 2012). Still, we would expect a combination: hormonal changes leading
461 to behavioral changes and these, in a kind of positive feedback, might then increase
462 hormonal differences.
463
464 **Conclusion**
465 Our results support the role of testosterone in important behavioral and physiological
466 changes associated with male dispersal. Up-regulation of testosterone seems to facilitate
467 dispersal-like behavior either through direct testosterone effects or, perhaps, through the
468 reduction of the reactivity of the HPA axis when philopatric males are coping with
469 dispersing opportunities. These two hypotheses are not mutually exclusive and both
470 involve testosterone actions. We previously demonstrated that an experimental increase
471 of testosterone levels caused juvenile philopatric group-living males to expand their home
472 ranges in African striped mice (Raynaud et al. 2012). However, the decision to disperse
473 relies on other factors; population density and reproductive competition are critical
474 predictors of dispersal in male African striped mice (Schradin et al. 2010b; Schoepf &
475 Schradin 2012). Thus, ecological factors may regulate testosterone signals which
476 facilitate behavioral, physiological, and morphological changes needed to disperse
477 (Raynaud et al. 2012) supporting the role of testosterone in dispersal.

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663 **Figure captions**

664

665 Figure 1. Serum hormone levels before (D0), one day (D1), nine days (D9), and 14 days
666 (D14) after the testosterone treatment in control (white columns) and test males (grey
667 columns); Figure 1a: testosterone; Figure 1b: corticosterone. The median is indicated by
668 the horizontal bar inside the box, the first and third quartiles by the box itself, outliers are
669 shown as black dots. *ns:* non significant; *: $p < 0.05$; **: $p<0.01$.

670

671 Figure 2. Percentage of time per day that alloparental care was shown by control (white
672 columns) and test males (grey columns): Total = huddling + licking + Nest; Nest = time
673 spent inside the nest with the pups. The median is indicated by the horizontal bar inside
674 the box, the first and third quartiles by the box itself, outliers are shown as black dots. *ns:*
675 non significant.

676

677 Figure 3. Figure 3a: time (s) away from the wall during the open field test; Figure 3b:
678 activity (%) of the test and control males during the open field test; Figure 3c: time spent
679 in the open arm (OTR; %) during the elevated plus maze test; Figure 3d: total number of
680 arm entries during the elevated plus maze test. The median is indicated by the horizontal
681 bar inside the box, the first and third quartiles by the box itself, outliers are shown as
682 black dots. Placebo (white columns) = control males; Testosterone (grey columns) = test
683 males; *ns:* non significant; *: $p < 0.05$.

684